PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2006) **361**, 2187–2198
doi:10.1098/rstb.2006.1939
Published online 6 November 2006

Oxytocin, vasopressin and pair bonding: implications for autism

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Understanding the neurobiological substrates regulating normal social behaviours may provide valuable insights in human behaviour, including developmental disorders such as autism that are characterized by pervasive deficits in social behaviour. Here, we review the literature which suggests that the neuropeptides oxytocin and vasopressin play critical roles in modulating social behaviours, with a focus on their role in the regulation of social bonding in monogamous rodents. Oxytocin and vasopressin contribute to a wide variety of social behaviours, including social recognition, communication, parental care, territorial aggression and social bonding. The effects of these two neuropeptides are species-specific and depend on species-specific receptor distributions in the brain. Comparative studies in voles with divergent social structures have revealed some of the neural and genetic mechanisms of social-bonding behaviour. Prairie voles are socially monogamous; males and females form long-term pair bonds, establish a nest site and rear their offspring together. In contrast, montane and meadow voles do not form a bond with a mate and only the females take part in rearing the young. Species differences in the density of receptors for oxytocin and vasopressin in ventral forebrain reward circuitry differentially reinforce social-bonding behaviour in the two species. High levels of oxytocin receptor (OTR) in the nucleus accumbens and high levels of vasopressin 1a receptor (V1aR) in the ventral pallidum contribute to monogamous social structure in the prairie vole. While little is known about the genetic factors contributing to species-differences in OTR distribution, the species-specific distribution pattern of the V1aR is determined in part by a species-specific repetitive element, or 'microsatellite', in the 5′ regulatory region of the gene encoding V1aR (*avpr1a*). This microsatellite is highly expanded in the prairie vole (as well as the monogamous pine vole) compared to a very short version in the promiscuous montane and meadow voles. These species differences in microsatellite sequence are sufficient to change gene expression in cell culture. Within the prairie vole species, intraspecific variation in the microsatellite also modulates gene expression *in vitro* as well as receptor distribution patterns *in vivo* and influences the probability of social approach and bonding behaviour. Similar genetic variation in the human *AVPR1A* may contribute to variations in human social behaviour, including extremes outside the normal range of behaviour and those found in autism spectrum disorders. In sum, comparative studies in pair-bonding rodents have revealed neural and genetic mechanisms contributing to social-bonding behaviour. These studies have generated testable hypotheses regarding the motivational systems and underlying molecular neurobiology involved in social engagement and social bond formation that may have important implications for the core social deficits characterizing autism spectrum disorders.

Keywords: individual differences; regulatory microsatellite; prairie vole; autism; vasopressin; oxytocin

1. INTRODUCTION

When Leo Kanner first described autism in 1943, he indicated that the main deficits were social withdrawal and lack of empathy, or in his words, an 'innate inability to form the usual…affective contact with people' (Kanner 1943). This can have devastating consequences for the emotional well-being of families with affected individuals, the rate of which is *ca* 1 per thousand individuals (Gillberg & Wing 1999). It is now clear that autism spectrum disorders are complex neurodevelopmental disorders likely involving many genetic and environmental interactions. Furthermore, these disorders are typically characterized by phenotypes beyond the disruptions in social engagement, including impairment in communication skills, and restricted, repetitive and stereotyped patterns of behaviour. While there are currently no animal models reflecting the broad range of the autism behavioural and neurological phenotypes, studies into the neurobiology of normal social cognition, engagement and bonding in animals may provide important clues useful for understanding the neurobiological mechanisms underlying the devastating social deficits in autism.

While it is true that the 'typical' social behavioural phenotype of humans includes not only social engagement, but also empathy and attachment to varying degrees, social bonding outside of the mother–infant

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One contribution of 14 to a Theme Issue 'The neurobiology of social recognition, attraction and bonding'.

bond is not typical in the vast majority of mammals. Social behavioural traits vary widely across and within species. This diversity in social behaviour can actually be harnessed as a tool to investigate the neural and the genetic components regulating normal social engagement and bonding that may be relevant to autism. One example illustrating the power of the comparative approach for understanding the neurobiology of social behaviour is the study of pair bonding in voles. Voles are hamster-sized rodents that vary widely in their social organization across species (Carter *et al.* 1995). Prairie voles are socially monogamous; males and females form long-term pair bonds, establish a nest site and rear their offspring together. In contrast, montane and meadow voles do not form bonds with a mate and only the females take part in rearing the young. These data were first obtained from extensive field studies and then brought into the laboratory in the 1980s (Getz *et al.* 1981). Based on earlier studies in rats, which demonstrated a role for the neuropeptide oxytocin in mother–infant bonding (Pedersen & Prange 1979; Kendrick *et al.* 1987), and vasopressin in social recognition (Dantzer *et al.* 1987; Le Moal *et al.* 1987), oxytocin and vasopressin systems were the initial candidate targets for exploration in these two species. Since then, both oxytocin and vasopressin systems have proved to be essential players in the regulation of social bonding in voles (Young & Wang 2004). Newer genetic techniques, such as viral vector gene transfer technology in combination with pharmacological manipulations have allowed for the testing of hypotheses about the regulation of social behaviour in voles as well as more conventional laboratory models such as mice and rats.

This review focuses on genetic and neural studies demonstrating the role of oxytocin and vasopressin in pair bonding in prairie voles. We also discuss relevant findings in more conventional laboratory models where appropriate. We emphasize the value of 'comparative neuroethological' and 'individual differences' approaches in understanding the neural bases of complex social behaviours. Additionally, we review the findings in humans which implicate oxytocin and vasopressin systems in human social behaviour, and the data suggesting their potential dysregulation in autism.

2. OXYTOCIN AND ARGININE VASOPRESSIN IN SOCIAL BOND FORMATION

Species with high levels of social-bonding behaviour must have in place neural mechanisms to reinforce or motivate its members to socially engage and bond. In the vole model, social bonding is part of a suite of behaviours associated with the monogamous social structure. As described previously, monogamy includes a long-term selective association with a partner throughout the breeding and non-breeding seasons and paternal contribution to care for the young (Carter *et al.* 1995). It is important to note that this definition of monogamy does not preclude sexual promiscuity. Additionally, prairie voles show selective aggression towards novel conspecifics after becoming sexually experienced (Winslow *et al.* 1993). Furthermore, pups of monogamous prairie voles, but not non-monogamous montane voles, show a robust stress response to maternal separation with increased vocalization and increased serum corticosterone levels (Shapiro & Insel 1990).

In the field, monogamy can be observed when male and female pairs are routinely trapped together and share nest sites (Getz *et al.* 1981). In the laboratory, monogamy can be quantitatively assessed by measuring several social behaviours such as: preference for a familiar partner, biparental care of offspring and selective aggression towards unfamiliar intruders. Most of the laboratory investigations of the neural basis of social bonding have used the partner preference assay to test for the presence of a pair bond (Williams *et al.* 1992*a*). It is important to note that the partner preference assay gives us a window into the development and maintenance of the pair bond, but it should not be confused with the pair bond itself.

In the laboratory, 'pair bonds' are created by the experimenter. While there is mate choice in the wild, in the laboratory, pairs are randomly assigned by the experimenter. Sexually naive males and females are paired for a cohabitation period. The longer the pair are together, the greater the likelihood of pair bond formation as measured by partner preference (Williams *et al.* 1992*a*). Mating during this period of cohabitation greatly facilitates the formation of partner preferences, although partner preferences can occur in the absence of mating. After a period of cohabitation, the animals are tested in a 3-h 'partner preference test', in which the preference of only one of the pair is tested. The testing chamber consists of three cages connected by Plexiglas tubes. The test subject is allowed access to all three cages. The test subject's 'partner' (from the cohabitation period) is tethered in one of the cages, a 'stranger' animal is tethered in a second cage and a middle connecting cage is considered 'neutral' and does not contain an animal. Both males and females can be tested in this apparatus. A 'partner preference' is operationally defined by the amount of time the test animal spends with the partner compared with the stranger. In most studies, the animals are considered to have a 'partner preference' when the test animal spends twice as much time in side-by-side social contact with the partner than with a stranger. Monogamous prairie voles show a preference for their partner in this behavioural paradigm (Williams *et al.* 1992*a*; Winslow *et al.* 1993). In contrast, non-monogamous meadow and montane voles do not show a preference for either animal (Insel & Hulihan 1995; Lim *et al.* 2004*b*). Instead, these non-monogamous animals often actually spend more time alone in the neutral cage compared to monogamous voles, although meadow voles have been observed to form pair bonds under certain circumstances (Parker *et al.* 2001). As mentioned above, prolonged cohabitation and mating both facilitate the development of partner preferences. These parameters can be altered to increase or decrease the frequency of partner preference behaviour in a given sample of prairie voles. For example, decreasing the length of the cohabitation period or preventing mating allows one to test the ability of pharmacological agents to facilitate the development of partner preferences. Likewise, extensions of the cohabitation period as well as confirming mating allow

one to test agents that can decrease the frequency of partner preference formation.

Oxytocin plays a very important role in several social behaviours such as social recognition, maternal behaviour and maternal–infant bonding (Pedersen & Prange 1979; Kendrick *et al.* 1997; Ferguson *et al.* 2001). Additionally, oxytocin infusions into the brain increased side-by-side contact and decreased aggressive behaviour in female prairie voles (Witt *et al.* 1990), and increased social contact in male rats (Witt *et al.* 1992) and squirrel monkeys (Winslow & Insel 1991). Using the partner preference test as described above with truncated cohabitation periods and no mating, chronic central infusion of oxytocin facilitated partner preference formation in female prairie voles (Williams *et al.* 1994). In the complementary experiment, a selective oxytocin receptor (OTR) antagonist chronically infused into the lateral ventricles inhibited partner preference formation in females that experienced prolonged cohabitation and mating with the male (Williams *et al.* 1994; Insel & Hulihan 1995). In both cases, the manipulation of oxytocin systems did not affect sexual behaviour; it selectively modulated partner preference behaviour.

These data demonstrate that central oxytocin and its receptor are involved in facilitating partner preference formation in female prairie voles; however, since these pharmacological manipulations were performed intracerebroventricularly, they do not indicate where in the brain oxytocin may be acting. Additionally, the data do not explain why some species display bonding behaviour and others do not, since it is believed that all mammals have genes encoding oxytocin and the OTR, as both are necessary for lactation (Nishimori *et al.* 1996).

Oxytocin is produced in the hypothalamus, including the supraoptic and paraventricular nucleus (Gainer & Wray 1994). Magnocellular neurons in these nuclei send projections to the posterior pituitary, where oxytocin is released into the bloodstream and has effects on parturition and milk ejection during lactation. Other cells in the paraventricular nucleus project to several forebrain limbic structures, the brainstem and spinal cord (Swanson & McKellar 1979; Swanson & Kuypers 1980). These sites of oxytocin synthesis and their projections are very highly conserved throughout mammalian species. In contrast, there are significant species differences in OTR distribution patterns among monogamous and non-monogamous vole species (figure 1*a*,*b*; Insel & Shapiro 1992). Some regions of the brain, including the caudate putamen and the nucleus accumbens (NAcc), have high densities of receptors in monogamous species compared with non-monogamous species. Oxytocin receptor antagonists applied directly to the NAcc or prefrontal cortex of female prairie voles inhibit mating-induced partner preference formation (Young *et al.* 2001), indicating that activation of OTRs in these areas of the brain is necessary for the development of partner preferences in prairie voles. The molecular mechanisms that lead to the species differences in OTR expression have not been elucidated, although several differences in putative transcription factor binding sites have been found between the OTR genes of prairie and montane voles (Young *et al.* 1996).

While oxytocin may also play a role in pair bond formation in males (Cho *et al.* 1999; Liu *et al.* 2001), the majority of studies on male, pair bond formation have focused on vasopressin. Vasopressin is involved in many social behaviours in males, including flank marking and aggression in hamsters (Albers & Bamshad 1998) and social recognition in rats and mice (Dantzer *et al.* 1988; Englemann & Landgraf 1994; Bielsky *et al.* 2004, 2005). Partner preference in voles requires prolonged cohabitation with a partner and is facilitated by mating. *In vivo* microdialysis studies suggest that vasopressin is released within the brain during mating (Morales *et al.* 2004). Indeed, infusions of vasopressin directly into the brain facilitate partner preference formation (without mating) and receptor antagonists block partner preference formation in male prairie voles (Winslow *et al.* 1993). As with oxytocin, all mammals studied to date have genes encoding the neuropeptide vasopressin and its primary brain receptor, the vasopressin 1a receptor (V1aR).

Vasopressin cell and fibre distribution patterns are highly conserved across species (Wang *et al.* 1996*b*). Several brain areas contain either vasopressinergic cells (cells expressing vasopressin mRNA and vasopressin immunoreactivity) and/or vasopressin immunoreactive fibres. Vasopressin production occurs in immunoreactive and mRNA-containing cells in the hypothalamus (suprachiasmatic nucleus, SCN; paraventricular nucleus, PVN; supraoptic nucleus, SON), the bed nucleus of the stria terminalis (BST) and the medial amygdala (MeA). Those sites of production send projections to brain areas where vasopressin immunoreactive fibres appear including the lateral septum (LS), ventral pallidum (VP), lateral habenular nucleus (LH), medial preoptic area (MPOA), BST, PVN and MeA (De Vries & Buijs 1983). Therefore, like oxytocin, vasopressin production, while localized to just a few brain areas, has the potential to affect receptors throughout the brain.

Vasopressin expression levels in brain areas outside the hypothalamus are sexually dimorphic: males have higher numbers of vasopressin expressing cells in the BST and more fibres in the LS and lateral habenula (De Vries & Buijs 1983; De Vries *et al.* 1983; De Vries 1990). Sexual dimorphism in these brain areas is regulated by gonadal steroids (De Vries *et al.* 1983), and the steroids have both organizational and activational effects (Wang *et al.* 1993). In rats, when neonatal males are castrated, they mature into adult males with female-like numbers of cells and fibres in the BST, amygdala and septum. In contrast, when castrated as adults, only the intensity of the immunoreactivity falls while cell numbers are maintained. In the prairie vole, when sexually naive adult males are castrated, levels of vasopressin immunoreactivity are reduced in cells and fibres of brain areas thought to be involved in paternal care in this species: cells of the BST and MeA and their projections to the LS (Wang & De Vries 1993). In contrast, vasopressin levels in hypothalamic regions of the brain (PVN, SCN, SON) are not altered by castration (Wang & De Vries 1993). Infusion of vasopressin into the LS of castrated males increases pup-directed behaviours and antagonist treatment decreases the same (Wang *et al.* 1994). Vasopressin

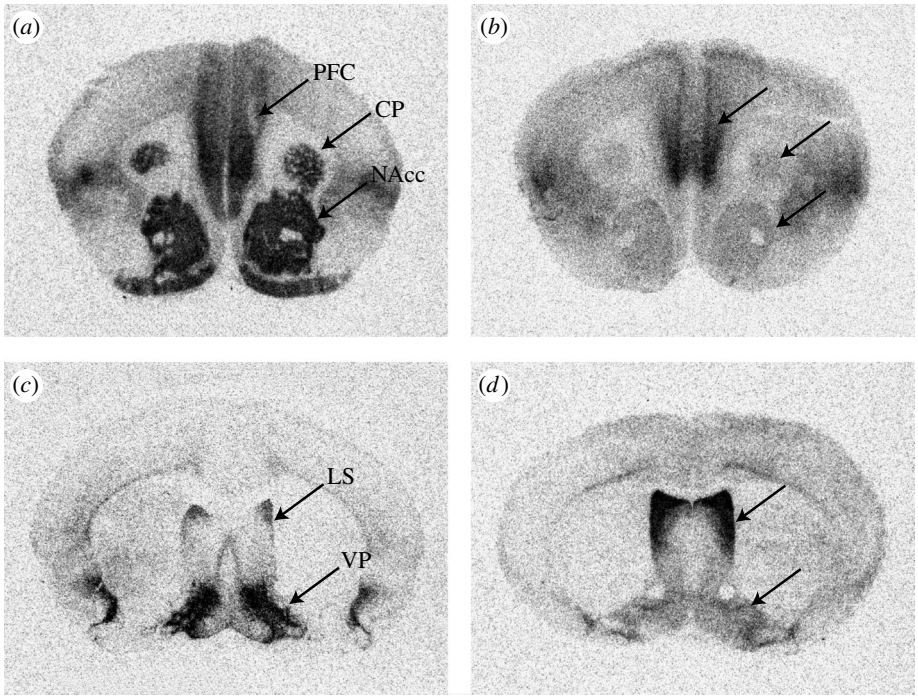

Figure 1. Autoradiograms illustrating the distribution of oxytocin receptors (*a*,*b*) and vasopressin receptors (*c*,*d*) in the monogamous prairie vole (*a*,*c*) and non-monogamous meadow vole (*b*,*d*). Note the species differences in oxytocin receptors in the NAcc and the vasopressin receptors in the VP. PFC, prefrontal cortex; CP, caudate putamen; NAcc, nucleus accumbens; LS, lateral septum; VP, ventral pallidum.

immunoreactivity is altered in male prairie voles after cohabitation with mating and pup exposure (Bamshad *et al.* 1993, 1994). Vasopressin immunoreactivity decreases in the LS with sexual experience and onset of parental care, while vasopressin mRNA levels rise in the BST (Bamshad *et al.* 1994). Since the vasopressin immunoreactive fibres in the LS originate from the BST, the coincidental decrease in LS vasopressin immunoreactivity and increased vasopressin mRNA in the BST are consistent with increased vasopressin release in the LS and a compensatory increase in vasopressin synthesis. In contrast to the findings presented above, castrated sexually naive prairie voles, with no vasopressin immunoreactivity in the LS, show only very mild deficits in spontaneous paternal behaviour (Lonstein & De Vries 1999). This indicates that vasopressin may not be required for the expression of paternal care, although it does not preclude a role for its modulatory influence.

Considering that vasopressin distribution is so highly conserved across species, it is interesting that it has such dramatic differences in behavioural effects when injected into the brain (Young *et al.* 1997, 1999). The brain distribution pattern for V1aR is not conserved even across closely related species like prairie and montane voles (figure 1*c*,*d*). Like the OTR, species differences in V1aR distribution have been hypothesized to contribute to the species differences in social structure (Insel *et al.* 1994; Young *et al.* 1997, 1999). In particular, the V1aR levels are higher in the VP, central nucleus of the amygdala, cingulate cortex and laterodorsal thalamus in monogamous prairie voles compared with promiscuous montane voles. In contrast to the sexually dimorphic levels of vasopressin peptide production outside of the hypothalamus, within the prairie vole species there do not appear to be any sex differences in the distribution patterns of V1aR (Insel *et al.* 1994; Phelps & Young 2003; Lim *et al.* 2004*a*). Interestingly, comparisons of other monogamous and non-monogamous species of rodents and primates revealed that high densities of V1aR in the ventral pallidal area are associated with monogamy (Young 1999). To test the idea that species-specific V1aR distribution patterns have important behavioural consequences, site-specific modulation of V1aR has been used. For example, a V1aR antagonist prevents partner preference formation when applied directly to the VP at low doses that are ineffective when delivered into the lateral ventricles (Lim & Young 2004). A viral vector containing the prairie vole *avpr1a* with a neuron-specific enolase promoter has been used to alter V1aR levels in specific brain regions. Increasing V1aR density in the VP of male prairie voles using this V1aR-expressing virus facilitates partner preference formation in the absence of mating (Pitkow *et al.* 2001).

If the species differences in V1aR levels in the VP are sufficient to alter species-typical behaviour, then experimentally increasing V1aR levels in the VP of promiscuous meadow voles should facilitate the development of partner preferences in this species. Lim and colleagues performed this experiment by injecting promiscuous meadow voles with a V1aR-expressing viral vector directly into the VP (Lim *et al.* 2004*b*). After injection and recovery, the animals were tested for their ability to form pair bonds in the partner preference test. All of the meadow voles that displayed increased levels of V1aR binding in the VP displayed partner preferences. Therefore, even though these two species diverged long ago, this simple change in the expression of a single gene replicated a hypothetical evolutionary event

that may have ultimately led to the development of monogamy.

Since both the NAcc and VP are key relay nuclei in the brain circuits involved in motivation and reward, it has been hypothesized that both oxytocin and vasopressin may be facilitating affiliation and social attachment in monogamous species by modulating these reward pathways (Insel & Young 2001; Young *et al.* 2001; Insel 2003; Young & Wang 2004). Dopamine in the NAcc plays a major role in the regulation of pair bond formation in prairie voles. After mating, dopamine turnover increases in the NAcc in both females (Gingrich *et al.* 2000) and males (Aragona *et al.* 2003). Dopamine D2 receptor antagonists in the NAcc block partner preference formation and agonists facilitate partner preference formation (Gingrich *et al.* 2000; Aragona *et al.* 2003). Concurrent activation of D2 dopamine and OTRs in the NAcc are necessary for partner preference formation in female prairie voles (Liu & Wang 2003). Additionally, the effect of increasing the V1aR levels in the VP on partner preference behaviour in the meadow vole depends on intact dopamine signalling, although this signalling has not yet been localized to the NAcc (Lim *et al.* 2004*b*). The VP and the NAcc are heavily interconnected (Zahm & Heimer 1990). Perhaps the activation of OTR in the NAcc of females and V1aR in the VP of males, along with concurrent activation of dopamine D2 receptors in 'reward circuitry' in both males and females provide sex-specific, but parallel mechanisms for pair bond formation in this species (Lim *et al.* 2004*a*).

Historically, dopamine innervation of the NAcc was thought to mediate the pleasurable sensations accompanying reinforcing stimuli. In contrast, newer theories of the role of dopamine along these pathways involve detection of cue saliency and perhaps calculation of costs and benefits, and more generally as a modulator of behavioural drive (Salamone *et al.* 2005). Regardless of the actual correlates of dopamine signalling, both theories of dopamine signalling are compatible with the idea that species-specific modulation of this pathway by oxytocin or vasopressin can influence the probability of partner preference formation. Therefore, if oxytocin and vasopressin, released during prolonged cohabitation and mating, modulate dopamine signalling in the NAcc in the prairie vole (but not in the montane or meadow vole) then both neuropeptides can alter the reinforcing properties of the mate and thereby influence the future behavioural interactions with the mate.

Clearly, the species differences in V1aR-binding patterns in the brain have important consequences for behaviour. These differences could be due to differences in receptor pharmacology across the two species or gene regulation. It appears that the latter is the case. The two species are highly homologous in the coding region for the *avpr1a* gene (Young *et al.* 1999) and consequently, there are no differences in the receptor pharmacology across the two species (Insel *et al.* 1994). Additionally, the species differences in receptor-binding levels are apparent at the perinatal period, indicating that environmental effects on regulation are less likely than genetic effects (Wang *et al.* 1996*a*). Furthermore, the species differences in distribution are not only apparent at the level of receptor binding, but also at the mRNA level, suggesting species differences in gene regulation rather than in post-translational processing (Young *et al.* 1997). In the regulatory region of the *avpr1a* gene, there is a striking species difference at *ca* 660 bp upstream of transcription start site. In monogamous prairie and pine voles, there is a 500 bp highly repetitive expansion, referred to as a microsatellite, at this locus which is only *ca* 50 bp long in the promiscuous meadow and montane voles (Young *et al.* 1999). It is possible that this microsatellite modifies gene expression patterns by changing the promoter structure of the *avpr1a* gene across monogamous and promiscuous species (Young *et al.* 1999; Hammock & Young 2002). A transgenic mouse for the prairie vole *avpr1a* gene, including this microsatellite, has a receptor distribution pattern which is more like that of a prairie vole than like a wild-type mouse, suggesting that sequences in the prairie vole *avpr1a* gene contribute to species-specific distribution patterns. In cell culture, this prairie-specific microsatellite modulates gene expression. Specifically, deleting the microsatellite results in an increase in the activity of a reporter gene in some, but not all, of the cell lines that were tested (Hammock & Young 2004). This indicates that the microsatellite acts in a cell-type-dependent manner to regulate gene expression. We would expect that such a regulatory mechanism is also functioning in the brain because not all brain areas show species differences in receptor-binding levels. In addition, when the prairie vole microsatellite is replaced with the montane vole microsatellite, the short montane vole microsatellite also increased reporter gene activity relative to the long prairie microsatellite, demonstrating that species differences in microsatellite length affect gene regulation (Hammock & Young 2004). It is plausible that the species differences in gene structure lead to changes in gene expression patterns, which ultimately have behavioural consequences.

Therefore, it does seem likely that the addition of the V1aR in the VP, by expansion or lack of contraction of this unstable repetitive element in the 5′ regulatory region of the *avpr1a* gene, was permissive for the natural selection of monogamous behaviour in the evolutionary history of prairie voles. If the instability of microsatellite sequences can serve as some sort of evolutionary tuning knob (King 1994), then there may still exist a genotype–phenotype relationship within the prairie vole species.

Within the prairie vole species, there is significant variability in the length of this microsatellite. There is also variability within-species in receptor distribution patterns and social behaviour (Hammock & Young 2002; Phelps & Young 2003). To test the hypothesis that the microsatellite serves as an evolutionary tuning knob, prairie voles were selectively bred for the length of their microsatellite and the parents and offspring were tested for their social behaviour and their distribution of V1aR in the brain determined (Hammock & Young 2005). First, it should be noted that subtle intraspecific variation in microsatellite length was able to modify gene expression in transcription reporter assays in cell culture, indicating that it might also function to regulate gene expression in some cell types in the brain. Second,

the paternal care of the breeder males, but not the breeder females, was associated with genotype. Specifically, breeder males with longer microsatellites spent more time in licking and grooming the pups, which is an important rodent parental behaviour that is known to be modulated by manipulating V1aR signalling (Wang *et al.* 1994). We tested the male offspring of the breeder pairs for their social behaviour and found that offspring with long microsatellites, as a group, were quicker to approach strangers and had higher rates of partner preference formation. The microsatellite appeared to have an effect on V1aR distribution patterns, as well. The effect of the microsatellite was region specific. A long allele appeared to increase V1aR levels in the olfactory bulb and LS and decrease in the hypothalamus, BST and several other brain regions. Interestingly, there were no differences in the VP and MeA, which are two brain regions that have been shown pharmacologically to play important roles in prairie vole typical social behaviour. Therefore, it seems as though unstable microsatellites may indeed play a critical role in the evolution of social behaviour by the generation of individual differences in brain and consequent behavioural traits.

Interestingly, a similar mutational event in the primate *AVPR1A* may have contributed to the evolution of primate social behaviour. Humans and bonobos, both known for high levels of social reciprocity, empathy and sociosexual bonding, have a repetitive microsatellite locus 3625 bp upstream of the transcription start site. In contrast, this microsatellite locus is absent in the common chimpanzee, reminiscent of the genetic differences between highly social and asocial voles at this locus (Hammock & Young 2005).

3. OXYTOCIN AND ARGININE VASOPRESSIN IN SOCIAL RECOGNITION

For social bonding to occur, individuals must have a capacity for social recognition, which includes the ability to detect as well as discriminate familiar individuals. In rodents, the main and accessory olfactory bulbs and their projections contribute significantly to this process. Oxytocin and vasopressin facilitate social recognition through these pathways. To facilitate social learning, these neuropeptides may act as more direct carriers of social information or may serve to modulate the activity of other neurotransmitter systems to increase the salience of social olfactory cues.

Social recognition requires a complex set of processes: social approach and investigation, sensory processing and learning and memory. Social recognition in rats and mice can be assessed experimentally owing to their high levels of novelty preference. Rats and mice will investigate a novel individual more than the one they have investigated recently. In tests for social recognition, the test animal is exposed to animal A and allowed to investigate for a brief period of time. This trial is followed by re-exposure to the same stimulus animal A or a novel stimulus animal B. With each exposure, the investigator records the duration of anogenital and perioral sniffing by the test animal. With repeated presentations of the same stimulus animal A, the test animal will spend less and less time investigating animal A. This decrease in the amount of investigation by the test animal is interpreted as social recognition, because now the familiar animal does not have novel properties. If the experimenter adds novel stimulus animal B to the test arena, then the test animal reverts to a thorough anogenital/perioral investigation behaviour. In this assay, social recognition memory has been determined to be *ca* 2 h or less for rats and mice (Dantzer *et al.* 1987), but can be prolonged or truncated with various behavioural and pharmacological manipulations. Behaviourally, group-housed mice display drastic improvements (days) in the duration of social memory (Kogan *et al.* 2000).

Oxytocin plays a role in social recognition. In rats, when infused into the olfactory bulb minutes prior to behavioural testing, oxytocin prolongs the duration of social recognition responses (Dluzen *et al.* 1998*a*). Oxytocin seems to modulate norepinephrine signalling in the olfactory bulb to enhance social recognition. Lesions of norepinephrine containing cells by intrabulb infusions of 6-hydroxydopamine prevent the prolonged social recognition response to oxytocin (Dluzen *et al.* 1998*b*). Norepinephrine seems to play a critical role in olfactory-mediated social recognition. Even without oxytocin coadministration, pharmacological induction of norepinephrine signalling in the olfactory bulb, by blockade of norepinephrine reuptake with nisoxetine or stimulation of alpha-2 noradrenergic receptors with clonidine, increases social recognition (Dluzen *et al.* 2000; Shang & Dluzen 2001). The emerging model is that oxytocin potentiates the release of norepinephrine in the olfactory bulb, since oxytocin treatment results in increased norepinephrine release as measured by microdialysis of the bulb (Dluzen *et al.* 2000). Oxytocin induced enhancement of norepinephrine stimulates alpha-2 noradrenergic receptors, which inhibit local inhibitory granule cells.

In addition to its role in the olfactory bulb, oxytocin appears to act in other brain areas to regulate social recognition behaviour. Infusions of oxytocin in both the LS and MPOA of male rats can prolong social recognition (Popik & Van Ree 1991, 1992). Male and female oxytocin knockout mice have an impaired ability to remember a mouse they have just met (Ferguson *et al.* 2000; Choleris *et al.* 2003). This deficit is rescued by infusion of oxytocin into the MeA before the learning trial, and in wild-type animals, the deficit can be reproduced with the application of an OTR antagonist into the MeA (Ferguson *et al.* 2001). The timing of treatment with oxytocin and its receptor antagonist indicate that oxytocin plays a role in the early stages of social-recognition memory formation rather than in the subsequent expression of social-recognition behaviour. Furthermore, mice with disruptions in the genes encoding oestrogen receptor alpha and beta, which are known to regulate the expression of OTR and oxytocin, also show social-recognition deficits (Choleris *et al.* 2003).

Like oxytocin, vasopressin is also involved in social recognition. Infusion of vasopressin into the brain can prolong the duration of social-recognition memory in rats (Le Moal *et al.* 1987). Vasopressin appears to act in the LS to mediate this affect, since vasopressin applied directly to the LS also prolongs the social-recognition

response, while a V1aR antagonist into the LS of rats inhibits social recognition (Dantzer *et al.* 1988). The Brattleboro rat, which cannot produce vasopressin, displays a social-recognition deficit, while application of vasopressin directly into the LS of these rats restores social recognition behaviour (Englemann & Landgraf 1994). Manipulating the numbers of receptors in the LS also influences social recognition behaviour in the predicted manner. For example, using V1aR selective antisense oligonucleotides to downregulate V1aR in the dorsal LS of normal rats inhibits social-recognition behaviour (Landgraf *et al.* 1995). Additionally, utilizing viral vector gene transfer of V1aR to increase V1aR density in the LS of normal rats enhances the apparent durability of social-recognition memories (Landgraf *et al.* 2003). Mice lacking the V1aR also have a social-recognition deficit (Bielsky *et al.* 2004) and this deficit is corrected with replacement of the receptor by viral vector gene transfer into the LS (Bielsky *et al.* 2005). Another brain receptor for vasopressin, the vasopressin 1b receptor (V1bR) also appears to be capable of influencing social recognition. The V1bR knockout also has a modest impairment in social recognition, although the primary phenotype is a reduction in aggression (Wersinger *et al.* 2002). In addition to its role in the LS, vasopressin also facilitates social recognition by acting in the olfactory bulb. Direct application of vasopressin to the olfactory bulbs prolongs the expression of the social-recognition response (Dluzen *et al.* 1998*a*), and as with oxytocin, the treatment effect may depend on intact noradrenergic signalling in the olfactory bulb (Dluzen *et al.* 1998*b*).

Like most mammals, rat pups are born helpless and evolution has developed mechanisms to ensure that the young receive the care they need. Rodent pups learn very quickly to associate received maternal care with sensory stimuli of the mother, most notably her odour. Rodent dams spend a lot of time licking and grooming the pup. This aspect of maternal care can be experimentally simulated using a paintbrush. When 'licked' with a paintbrush during exposure to a novel odour such as peppermint, rat pups will show a learned preference for peppermint odour that persists for some time after training (Sullivan *et al.* 1989). Therefore, maternal licking enhances olfactory learning in the pup. There appears to be a critical period for this kind of learning and it is associated with increased activity of the developing locus coeruleus, the major site of norepinephrine production. Rat pups have increased noradrenergic neurotransmission from the locus coeruleus into the olfactory bulb relative to adults (Sullivan *et al.* 2000; Sullivan 2003). In fact, this learning of and subsequent preference for an odour can be reproduced with exogenous application of norepinephrine into the bulb or stimulation of the locus coeruleus (Sullivan *et al.* 2000).

Perhaps in rat pups, oxytocin and/or vasopressin potentiates the release of norepinephrine to modulate social memory as oxytocin does in adult rats (Dluzen *et al.* 2000). Normal rat pups learn to associate a non-social odour (such as lemon scent) with the odour of their mom and will subsequently show a preference for the lemon-scent even in the absence of their mother. In contrast, pre-weanling vasopressin-deficient Brattleboro rats do not make the association of mother odour cues with a non-social odour, or at least fail to show a preference for the lemon odour after repeated mom-odour pairings (Nelson & Panksepp 1998). Likewise, in normal pre-weanling rats, intracerebroventricular OTR antagonist administration prior to the learning trial inhibits the association of mother odour with a non-social odour (Nelson & Panksepp 1996). Therefore, an emerging model of social olfactory learning in the rodent pup includes increased noradrenergic drive to the olfactory bulb which is potentiated by oxytocin and or vasopressin released with social stimuli. This increased noradrenergic drive probably reduces the inhibitory tone on the olfactory bulb, allowing increased activity of the main output cells of the bulb. This increased excitation in the output cells of the bulb probably results in plasticity in both the bulb and its downstream projections. Individual differences in developmental plasticity of the olfactory bulb may impact adult sociobehavioural phenotypes by differential tuning of the system to attend differently to social odours.

In rats and mice, it is very clear that appropriate social behaviour requires an intact sensory modality (olfaction) for social recognition, a way to assess the valency of the olfactory input (as in the amygdala) and regulation of motivation and reward circuitry (ventral forebrain). This circuitry is influenced by steroid hormones, neuropeptides and classical neurotransmitters like norepinephrine and dopamine. This is clearly a very simplified model as there are many other brain regions containing many hormones and gene products that contribute to the regulation of the many aspects of complex affiliative behaviour.

Interestingly, while there are clear data that suggest a role for vasopressin and oxytocin in social-recognition memory in rats and mice, the role of these neurohormones in the requisite social-recognition component of pair bonding behaviour in voles is not known. What little we do know about general vole social recognition does fit into the theoretical framework generated by rat and mouse studies. Lesion studies in female prairie voles have demonstrated that the main and accessory olfactory bulbs are critical for partner preference formation (Williams *et al.* 1992*b*; Curtis *et al.* 2001). In male prairie voles, lesions of the olfactory bulb result in decreased social behaviour (Kirkpatrick *et al.* 1994).

4. OXYTOCIN AND ARGININE VASOPRESSIN IN HUMAN SOCIAL BEHAVIOUR

As stated at the outset, we propose that understanding the neurobiological mechanisms regulating normal social behaviour in animal models will provide valuable clues to the regulation of human social behaviour, and perhaps suggest how dysregulation of these systems might contribute to developmental disorders such as autism. We are only at the very earliest stages of understanding the genetics and neural circuitry of complex social behaviours. However, ultimately it is hoped that these approaches will lead to novel pharmacological therapies that may ameliorate at least the social behavioural deficits in autism and Asperger's syndrome. The studies outlined above, as well as the

examples discussed below, make a very strong case in support of the hypothesis that oxytocin and vasopressin may modulate human social behaviour. However, at this point, a strong argument for a role of oxytocin and vasopressin in autism cannot be made, and that is not our intent here. With that disclaimer, we will discuss some intriguing data consistent with the hypothesis that disruptions in oxytocin and vasopressin systems may contribute to the behavioural phenotype of autism.

One important caveat when trying to link the animal literature on oxytocin and vasopressin discussed above to human behaviour is that the majority of the social behaviours modulated by these peptides in animals rely on olfactory projections to subcortical areas. In contrast, social cognition in humans involves visual and auditory sensory processing as well as attentional and executive areas of the cortex. However, it is possible that these neuropeptides modulate limbic areas in humans which receive multimodal sensory and cortical, rather than primarily olfactory, input. This is particularly plausible given the evolutionary plasticity in the neuroanatomical distribution of oxytocin and vasopressin receptors (figure 1) across mammalian species. Studies examining the effects of oxytocin and vasopressin on visual social cognition in humans would be useful for examining this possibility.

The distribution of the human OTR is consistent with an interaction with the dopamine system (Loup *et al.* 1991), with receptors concentrated in the substantia nigra, globus pallidus and nucleus of Meynert. Interestingly, a functional magnetic resonance imaging study suggests a high degree of overlap between OTR distribution and brain activation while viewing romantic partners or in mothers viewing images of their infant (Bartels & Zeki 2004). Plasma oxytocin levels have been reported to increase both during sexual intercourse and during breastfeeding in humans (Carmichael *et al.* 1987; Unvas-Moberg 1998). More recent studies have directly tested the influence of oxytocin on social behaviour by using intranasal infusions. First, intranasal infusion of oxytocin appears to enhance the buffering of social support in humans, as indicated by decreased stress response during a socially stressful situation (Heinrichs *et al.* 2003). A more recent study found that intranasal infusion of oxytocin increased trust among humans (Kosfeld *et al.* 2005).

There is modest, yet intriguing evidence linking oxytocin to autism. Oxytocin levels in blood plasma of boys with autism was found to be lower than in a group of age-matched controls (Modahl *et al.* 1998). Within the control group, oxytocin levels were positively associated with measures of social behaviour such as socialization, social coping and interpersonal relationships as well as some non-social measures like personal care and daily living skills. In contrast, oxytocin levels within the autistic group were negatively correlated with interpersonal relationships, socialization, community skills, personal care and daily living skills in addition to other behaviours. The oxytocin levels did show some overlap between the autistic group and the control group. The opposite directions of the correlations of oxytocin and behaviour mean that autistic kids with the most normal oxytocin levels had the worst social phenotype. This makes the data difficult to interpret and the potential role of oxytocin in the aetiology of autism very unclear. It is possible that the differences in oxytocin levels could reflect differences in medication between the groups. Additionally, the entire study is based on serum measures of oxytocin rather than measures from cerebrospinal fluid, which adds a layer of complexity as well. A follow-up study on these same samples revealed that the differences in plasma oxytocin levels were associated with an increase in incompletely processed oxytocin fragments, suggesting that peptide processing may be dysregulated in the autistic patients (Green *et al.* 2001). A second study has replicated these initial findings in a separate population of subjects (Al-Ayadhi 2005). This study also found lower levels of vasopressin in the plasma of autistic children. No studies have yet examined the pharmacological influences of oxytocin on the social deficits in autism; however, infusions of synthetic oxytocin and pitocin significantly reduced repetitive behaviours in patients with autistic and Asperger's disorders (Hollander *et al.* 2003).

While the plasma oxytocin data are consistent with the hypothesis that disruptions in the oxytocin system contribute to the social behavioural phenotype in autism, it is also equally plausible that these differences in oxytocin levels may be the consequence of altered cognitive processing in autistic patients. Autism is also characterized by general cognitive impairments, altered sensory processing and also cortical and cerebellar development, each of which are likely independent of the oxytocin and vasopressin systems. The altered processing of social stimuli resulting from altered brain wiring may actually prevent the normal activation of the oxytocin and vasopressin systems resulting in decreased plasma concentrations.

However, there is some modest evidence suggesting a possible association of the OTR gene with autism. A combined analysis of the primary genome scan data of the autism genetic resource exchange Finnish autism samples identified chromosome locus 3p24–26 as a candidate autism locus (Ylisaukko-Oja *et al.* 2005). This region contains 40 genes, including the OTR gene. However, this study failed to identify an association between specific polymorphisms and autism, although a limited number of polymorphisms were analysed. However, a separate study did report a significant positive association of the OTR gene with autism in a Chinese Han population (Wu *et al.* 2005).

For vasopressin, the most intriguing data suggesting a potential role for autism comes from genetic studies of the *AVPR1A* gene. There are three polymorphic microsatellites in the 5′ flanking region of the human *AVPR1A* (Thibonnier *et al.* 2000). Using a sample of 115 trio families, Kim *et al.* (2001) reported a nominally significant transmission disequilibrium between a microsatellite in the 5′ flanking region of the human *AVPR1A* and autism. This microsatellite is located 3625 bp upstream of the transcription start site and consists of a complex repeat of $(CT)_4$-TT-$(CT)_8$-$(GT)_n$ where n ranges from 9 to 25, resulting in 16 different alleles in the population. It should be noted that the transmission disequilibrium in this study fails to be significant if Bonferroni corrected for all of the

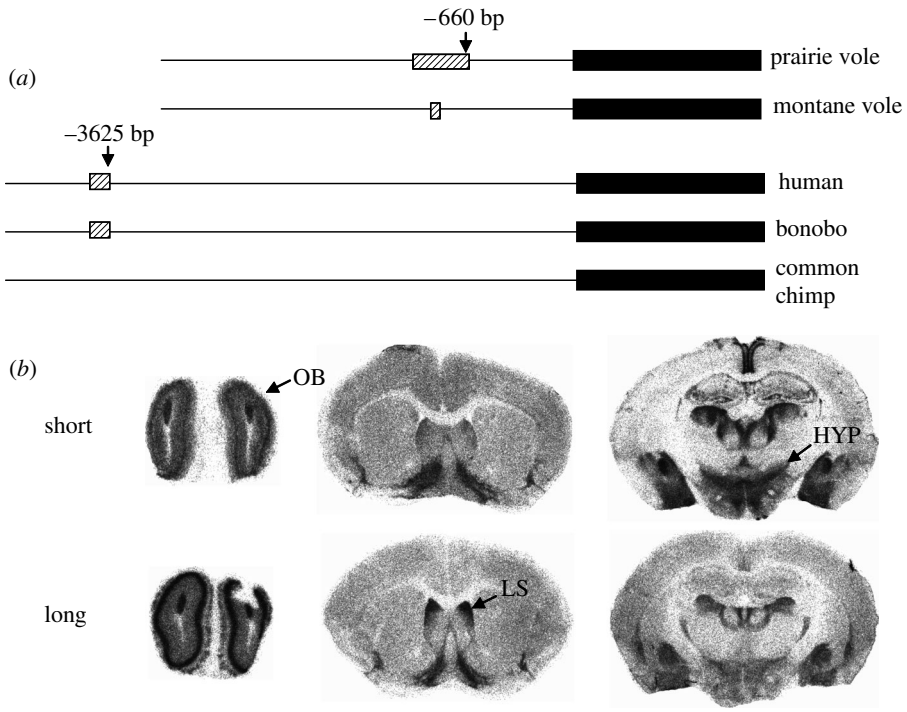

Figure 2. (*a*) Schematic of the structure of the vasopressin receptor gene *avpr1a* in voles and *AVPR1A* in primates. The black bars represent the transcribed region of the gene. The hatched bars represent the microsatellite sequences in the 5′ flanking region of the gene as discussed in the text. Numbers above the microsatellites indicate the relative position upstream of the transcription start site. (*b*) Autoradiograms illustrating the differences in vasopressin receptor binding in prairie voles with either short (top row) or long (bottom row) versions of the microsatellite in the *avpr1a* gene. Note the strain differences in the olfactory bulb (OB), lateral septum (LS) and the hypothalamus (HYP).

genes examined in this set of trios; therefore these data must be viewed with caution. However, it should also be noted that given the estimation that several dozen genes may be involved in the aetiology of autism, stronger associations may not be expected. The association between this microsatellite and autism was recently replicated by a second independent study (Wassink *et al.* 2004). Interestingly, this study found the strongest transmission disequilibrium in the subset of autistic children without language impairment. While the chromosomal locus of *AVPR1A* has not been identified in genome scans as a candidate locus in autism, one study in which only autistic subjects with normal language function were analysed, a chromosomal locus containing *AVPR1A* was identified as a candidate site. Therefore, while variation at this locus alone is not likely to cause autism and may not be associated at all with some forms of autism, variation may contribute to the severity of disease phenotype in some individuals, or potentially interact with other genetic or environmental factors that more directly cause autism.

Interestingly, this same polymorphic microsatellite in the human *AVPR1A* that has been associated with autism is absent in the common chimpanzee, but present in the bonobo (Hammock & Young 2005). Bonobos are known for their high levels of sociosexual reciprocity and they appear to use sexuality to promote social reconciliation as well as social bonding within their group (De Waal & Lanting 1997). Therefore, it is intriguing to consider that as in voles, variations in unstable microsatellite sequences in the promoters of the primate vasopressin receptor gene (figure 2) may contribute to species differences in expression and social behaviour, as well as to individual differences in social behaviour.

5. CONCLUSION

Basic research into ethologically relevant behaviour of the prairie vole has allowed us to gain insight into some of the underlying neural and genetic mechanisms of social-bonding behaviour in mammals. Humans may share some of these mechanisms and when these mechanisms are disrupted, either by genetic, environmental or interactive causes, extreme phenotypes such as autism may be revealed. These studies illustrate the power of the comparative neuroethological approach for understanding human neurobiology and suggest that examining the neurobiological bases of complex social behaviour in divergent species is a valuable approach to gaining insights into human pathologies.

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